

Complex preparation and crystallization

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An abbreviated version of this protocol was published in mAbs in Apr 2019

Glycosylation-independent binding of monoclonal antibody toripalimab to FG loop of PD-1 for tumor immune checkpoint therapy

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Detailed protocol

The mammalian cell expressed PD-1 protein and toripalimab-Fab fragment were mixed at a molar ratio of 1:1. The mixture was incubated on ice for 30 min and further purified by SuperdexTM 200 10/300 GL (GE Healthcare). 10 mg/mL of toripalimab/PD-1 proteins were used for crystal screening by vapour-diffusion sitting-drop method at 4°C. Diffractable crystals were obtained in a condition consisting of 0.09 M Halogens consisting of NaF, NaBr and NaI additives, 0.1 M Tris-Base (pH8.5), 37.5% (v/v) MPD-P1K-P3350 consisting of MPD (racemic), PEG 1K and PEG 3350 (Morpheus[®] MD1-46 kit, Molecular Dimensions).

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1. Tan, S. , Feng, H. and Yan, J. (2021). Complex preparation and crystallization. Bio-protocol Preprint. bio-protocol.org/prep1258.
2. Liu, H., Guo, L., Zhang, J., Zhou, Y., Zhou, J., Yao, J., Wu, H., Yao, S., Chen, B., Chai, Y., Qi, J., Gao, G. F., Tan, S., Feng, H. and Yan, J. (2019). Glycosylation-independent binding of monoclonal antibody toripalimab to FG loop of PD-1 for tumor immune checkpoint therapy. mAbs 11(4). DOI: [10.1080/19420862.2019.1596513](https://doi.org/10.1080/19420862.2019.1596513)

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